# Sex pheromone communication in the Lepidoptera: New research progress

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*Summary.* Significant progress has been made recently in research on lepidopterous sex pheromones. Advances in understanding the biochemical, neurobiological, and behavioral events that result in both successful and unsuccessful pheromone communication have allowed researchers to gain new insights into the genetic control and evolution of pheromone systems.

*Key words.* Sex pheromone; biosynthesis; neurobiology; sensory biology; orientation behavior; flight control; anemotaxis; genetics; communication; olfaction; evolution.

## *Introduction*

In recent years, research on sex pheromones in the Lepidoptera has continued to advance our knowledge along several fronts, and has opened new frontiers in several areas. Sex pheromones have traditionally been considered by researchers to be those compounds that are emitted by individuals of one sex and which cause attraction of members of the opposite sex, resulting in the location of the emitter, and subsequently, mating. Attraction is not necessarily the only behavioral effect that characterizes sex pheromones. Elicitation of courtship behavior in the attracted sex, among other things, may also be included. However, attraction, especially over long distances (ca 1 m or more)[44] is the predominant effect of these compounds, and for over a hundred years this attraction has fired the imagination of scientists and naturalists, due to the distance over which attraction can occur, the amazing specificities of the signals, and the enormous sensitivities of the receivers.

Most of the recent advances have occurred at the organismal, cellular, and molecular levels of inquiry. This is not to say that significant, even profound progress on the effects of pheromones on populations has not occurred, both on basic[64] and applied ecological fronts[77]. Also, new chemical identifications of pheromone blends have continued unabaited[83] – this task is still not nearly as easy as people outside of the field seem to think – to provide the powerful tools possessed uniquely by the field of lepidopterous sex pheromone research for understanding chemical communication and olfaction. Nevertheless, because there has been a recent explosion of significant pheromone research at the behavioral, physiological, and biochemical levels, these areas will be emphasized in this paper.

## *Biochemistry*

### *Pheromone biosynthesis*

In the past 8 years or so great strides forward have been made toward understanding how female moths biosynthesize most of the known pheromone components used, especially by those species in the Tortricidae, Noctuidae, and Pyralidae. As pointed out by Bjostad et al.[18], most of these components have the following characteristics[83]. First, they are straight-chain structures, with an even number of carbon atoms 10–18 carbons in length. Second, they have a functional group on the end of the molecule that is either an aldehyde, alcohol, or acetate. Third, they have either one, two, or three double bonds at various positions on the molecule in either the *Z* or *E* geometric configuration.

Only since the pioneering work of Bjostad, Roelofs and colleagues beginning in 1981 have the details of the biosynthesis of these structures been uncovered. It is now clear that the great diversity of the structures in the tortricids, noctuids, and pyralids are biosynthesized by means of a surprisingly few biochemical reactions. The routes to a smaller set of compounds used mostly by the Arctiidae, Lymantriidae, and some subfamilies of Noctuidae are largely unexplored. Such compounds are branched and unbranched straight-chain hydrocarbons with no functional group, sometimes saturated but usually multiply unsaturated at the 1, 3, 6, 9, 12 or 15 positions in the *Z* configuration, or with an epoxide instead of a double bond at these positions[83].

Two species, *Argyrotaenia velutinana* and *Trichoplusia ni*, offer the most complete picture of what occurs in the Tortricidae and Noctuidae, respectively. In the redbanded leafroller moth, *A. velutinana*, a complex blend of seven components is used by females for optimal attraction of males[16]: (*Z*)-11-tetradecenyl acetate (*Z*11–14:Ac), (*E*)-11-tetradecenyl acetate (*E*11-14:Ac)[78], tetradecyl acetate (14:Ac), dodecyl acetate (12:Ac), (*Z*)-9-dodecenyl acetate (*Z*9-12:Ac), (*E*)-9-dodecenyl acetate, (*E*9-12:Ac) and 11-dodecenyl acetate (11-12:Ac)[16]. Radiolabelling studies showed that the main chain is synthesized de novo from acetate, with significant incorporation of radiolabelled acetate into tetradecanoate, hexadecanoate, and octadecanoate in the lipids extracted from the pheromone gland. Longer fatty acyl molecules did not exhibit much incorporation. To get the tetradecanoate chain needed for the major pheromone components, a variety of experiments indicated that the predominant route used by females is a chain-shortening of hexadecanoate by means of beta-oxidation. The double bonds in the 11 position were found to be formed by the activities of both an *E* and a *Z*-11 desaturase acting on

the tetradecanoate (after the chainshortening of hexadecanoate)[17]. Among the evidence was that incubation of glands with [1,2,3-$^{13}$C] tetradecanoic acid resulted in three extra mass units incorporated into 14:Acyl, *Z*11-14:Acyl, *E*11-14:Acyl, 14:Ac, *Z*11-14:Ac, and *E*11-14:Ac; and incubation of [1,2,3,4-$^{13}$C] resulted in four extra mass units incorporated into these same compounds. Incorporation of label into unsaturated fatty acyl groups with 16 or 18 carbons was not observed. The presence of a glandular isomerase acting in conjunction with a *Z*-11 desaturase to form the *E*11-14:Ac was ruled out by various experiments with isomerically pure labelled precursors and no formation of the opposite isomer[17,18].

When no isomerase was found, just how the precise 92:8, female-produced *Z*:*E* ratio is produced became a puzzle, especially when no such ratio was found in any of the fatty acyl precursors. By examining the *Z*:*E* ratio in many different lipid classes, the evidence strongly suggested that large amounts of both isomers are formed by the activities of their respective *E* and *Z*11-desaturases. Then the gland apparently preferentially selects about 10 times as much *Z*11 as *E*11 isomer from phospholipids, the class of lipids that likely serve as early intermediates for these fatty acyl groups after desaturation[18]. The gland first reduces, then acetylates these selected 14:Acyl molecules to form the 92:8 *Z*11: *E*11-14:Ac ratio that the female emits. The rest of the *Z* and *E*11-14:Acyl groups in the phospholipids that are not used for forming the pheromone components then appear to be converted to triacylglycerols, which in effect form a dump for the unselected 14:Acyl groups[18]. The pool of 14:Acyl precursors in the phospholipids from which the gland can select *E* and *Z* isomers thus never would become skewed with too much *E*, and thus would remain a fresh source of a constant ratio of *E* and *Z* molecules. Evidence for this route included the observation that although radiolabel is readily incorporated into the triacylglycerols, no labelled triacylglycerols were ever observed to contribute to the formation of pheromone components[17]. Also, the 1:2 *E*:*Z* ratio found in the phosphatides was consistent with the phosphatides being the early intermediates in *E*:*Z* pheromone component ratio formation.

During these studies, it became clear that although *Z*11 and *E*11-14:Ac remained as the most important pheromone components of this species' blend, other minor components (see above) also significantly influenced upwind flight when included in the blend[16]. Most of these components are 12-carbon acetates that can be accounted for either by an extra chain-shortening step following Δ11 desaturation (*Z*9-12:Ac), Δ11 desaturation following the extra chain-shortening step (11-12:Ac), or no desaturation at all, combined with two chain-shortening steps from hexadecanoate to first form 14:Ac (a fifth pheromone component) and then 12:Ac (a sixth component) (fig. 1). Of course reduction and acetylation is the final step in all of these pathways (fig. 1).

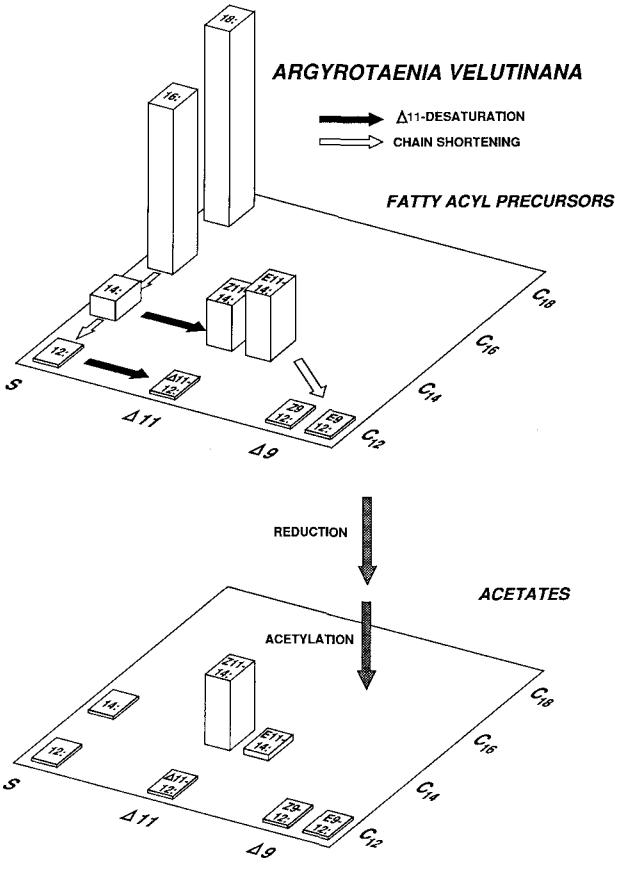


Figure 1. Proposed pathways involved in the biosynthesis of the sex pheromone components of the redbanded leafroller moth, *Argyrotaenia velutinana*. Reproduced from Bjostad et al.[18], by permission of Walter de Gruyter, Inc.

These same types of enzymes were shown to be used in the formation of the pheromone blend of the cabbage looper *Trichoplusia ni*[14,94], except that only *Z*11, not *E*11-desaturase, was found, accounting for the lack of *E* isomers in any of the pheromone components[15]. Another major difference between *T. ni* and *A. velutinana* was that for the majority of components, the first desaturation occurs before chain-shortening of either hexadecanoate or octadecanoate, and the various minor components then arise by reiterative chain-shortening of the molecules to form a homologous series of *Z* isomers on 12- or 14-carbon chains (fig. 2)[16,18].

Insight into the terminal enzymes in the sequence that create behaviorally active acetate, alcohol, or aldehyde molecules in moths has come from experiments by Teal and Tumlinson[84]. They found that the alcohols in the gland of *Heliothis virescens* and *H. zea* (Noctuidae) are oxidized to the aldehyde pheromone components just prior to emission from the gland surface by a nonspecific alcohol oxidase in the gland cuticle. They added large amounts of synthetic alcohols to the gland surface, then extracted the gland and found that correspondingly aberrant, high ratios of the aldehyde pheromone components corresponding to that alcohol chain length and unsatura-

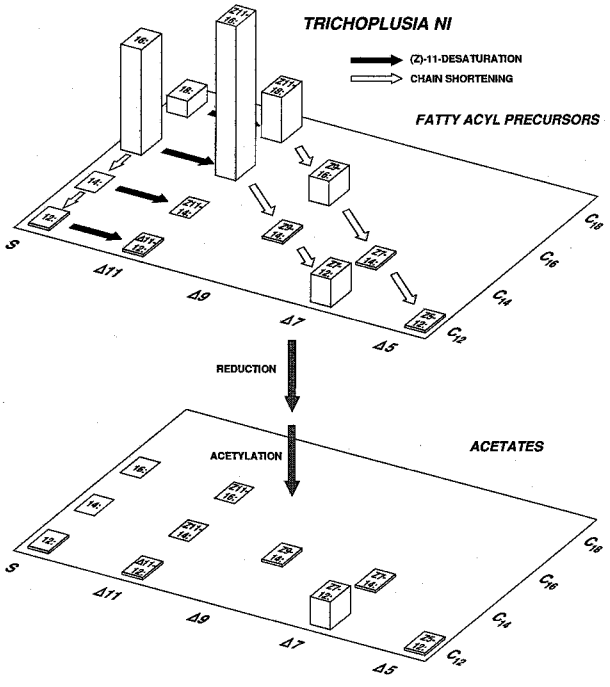


Figure 2. Proposed pathways involved in the biosynthesis of the sex pheromone components of the cabbage looper moth, *Trichoplusia ni*. Note how the initial step towards production of all components is (*Z*)-11 desaturation, whereas in *A. velutinana*, the first step is chain-shortening (fig. 1). Reproduced from Bjostad et al.[18], by permission of Walter de Gruyter, Inc.

tion had been synthesized. Interestingly, by applying bombykol to the surface of *H. virescens* glands, females were created that synthesized the alien aldehyde, bombykal [(*E*)-10, (*Z*)-12-hexadecadienal], in large amounts. Also, by doping the *H. zea* glands with *Z*9-14:OH and 14:OH, they created calling *H. zea* females that strongly attracted *H. virescens* males in a flight tunnel. This was due to the fact that the *Z*9-14:OH and 14:OH were oxidized to *Z*9-14:Ald, and when these compounds are added to the four known 16-carbon aldehydes of the *H. zea* pheromone, *Z*11-16:Ald, *Z*9-16:Ald and 16:Ald, the blend of *H. virescens* is created[53, 54, 85]. *H. virescens* males will not fly upwind to the 16-carbon aldehyde components without *Z*9-14:Ald present[85, 87]. The enzyme would not convert alcohols to aldehydes in the absence of molecular oxygen, and although it was relatively unspecific for carbon chain length, it would not convert secondary alcohols to aldehydes. Thus in these species relatively large amounts of fatty acyl groups appear to be reduced one step to alcohols and stored as alcohols until just before pheromone emission occurs, when a cuticular-bound primary alcohol oxidase creates the aldehyde pheromone components during female calling[84].

The power of knowing that merely changing the sequence of action of relatively few enzymes accounts for most of the pheromone molecules in the Lepidoptera should not be underestimated. This knowledge has not only facilitated the identification of behaviorally important minor components in new pest species due to the solid predictions it allows but it has also sharpened our insight into the possible evolutionary pathways that have shaped lepidopteran sex pheromone communication. Roelofs and Brown[80] were able to construct a phylogenetic model for the Tortricidae in which the combined knowledge about pheromonal biochemical pathways and traditional morphological features gave a consistent picture of the worldwide relationships among the subfamilies and tribes in this family. Several other enzymes than those mentioned above need to come into play, however, to account for all the known tortricid pheromones. Double bonds at even-numbered carbons are accounted for by a $\Delta$10- or $\Delta$8-desaturase[80]. Multiple double bonds are accounted for by the desaturases acting first before, and then also after, chain-shortening[80]. In addition, the ubiquitous *Z*9-desaturase that occurs throughout the plant and animal kingdoms[18] seems to come into play in some systems, and there is now also evidence for yet another type of desaturase, *E*9-desaturase[61].

Recently, it has become clear that some conjugated double bond systems may be created not by the reiterative action of a desaturase both before and after chain-shortening[80], but also by a desaturase putting one double bond in place followed by a 1,4-desaturation[18, 61]. For instance, in the silkmoth *Bombyx mori*, the presence of large quantities of *Z*11-16:Acyl groups in the gland supports the scenario that *Z*11-desaturase first acts on hexadecanoate, then undergoes 1,4-desaturation to create the conjugated diene system in the *E*10, *Z*12-16:Acyl molecule which is then reduced to the pheromone alcohol and oxidized to aldehyde[18]. Löfstedt and Bengtsson[61] are more specific in outlining how the 1,4-desaturation might proceed in creating the conjugated diene system in the codling moth, *Cydia pomonella*. They found evidence that *E*9-desaturase first acts on the 12:Acyl group. The *E*9 double bond system would undergo oxidation at one of the alpha positions surrounding the double bond, followed by the 1,4-elimination of water to create the *E*8,*E*10-12:Acyl group that is then reduced to the alcohol pheromone component. This proposed novel enzymatic pathway to the conjugated diene used by *C. pomonella* is of no trivial importance. The phylogenetic distances among different tortricids will be determined by species' usages of different vs common enzyme systems, and of course these enzymes are the products of genes that have been naturally or sexually selected over evolutionary time.

*Pheromone reception*

A new model has recently emerged for how pheromone migrates from the surface of a sensory hair on a male's antenna to the receptor site on a sensory neuron and then is deactivated[90, 91]. Previous models had been formed based on electron micrographs that showed that some

pore tubules leading into the hair from the outside appeared to have direct access to the dendritic surface, i.e., that the outside air had direct contact with the receptor cell[39,91]. Thus the pheromone would diffuse down the pore tubule, interact with the dendritic receptor site, and then be cleared away from the site into the sensillum liquor by the combined action of a rapid, non-enzymatic early inactivation process and a slower-acting process of pheromone degradation by means of enzyme[39,40].

The recent kinetic equilibrium model by Vogt and Riddiford[90] and Vogt[91], on the other hand, focuses on the majority of tubules that in electron micrographs seem to fall short of the dendritic surface. This model proposes that after reaching the end of the pore tubule, the incoming pheromone must traverse the sensillum liquor in order to reach the dendrites of the olfactory cells. This would be a difficult feat because the liquor is aqueous and thus thought to be highly lipophobic. Vogt and Riddiford[89] found, though, that the concentration of pheromone binding protein in the liquor of the silkmoth *Antheraea polyphemus* was very high, on the order of 20 mM, which approximates a 30% solution. They also found that although the affinity of the binding protein for pheromone was very low, at high enough concentrations of binding protein the association of pheromone with the population of protein molecules becomes strong enough that the protein becomes an effective solubilizer, carrier, and protector of pheromone. Thus they proposed that it would be possible for pheromone to move across the sensillum liquor in much the same way as it chromatographs on a gas-liquid chromatograph, with the binding protein acting as the active, stationary phase. Because the binding constant is so low, the pheromone will spend very little time on any one protein molecule, but because there are so many of the molecules, the mass action would result in the migration of the pheromone across the liquor to the receptor sites on the dendrites.

A second component of the model is that enzymatic degradation of pheromone is the principal means of rapid early inactivation of pheromone once it has reached the receptor sites. Although degradative enzyme molecules, in this case antennal pheromone esterase[88,89], are at a very low density in the liquor relative to binding protein, their high pheromone binding constant would make them extremely effective at clearing the liquor of active acetate pheromone molecules by changing them to alcohols. Both the pheromone binding protein and antennal esterase were shown to be housed only in the antennal sensilla of males, not anywhere else on the body of adult moths or on female antennae. The estimated half-life of pheromone in the presence of only the isolated esterase was ca 15 ms[90,91]. In the presence of binding protein the half-life would be significantly longer. Previous estimates of pheromone half-lives were on the order of 4 min, and the major portion of the early inactivation[39] or clearing away of pheromone from receptor site was envisioned to occur mostly by means of a non-enzymatic process such as the binding protein[39,40]. The kinetic equilibrium model hypothesizes that some pheromone might be degraded before it ever reaches a receptor site. Some pheromone would reach the receptor site via binding protein, then be removed by binding protein and finally encounter an esterase molecule and be transformed into an inactive alcohol. Other pheromone molecules might reach the receptor site, leave and chromatograph across more binding protein to another receptor site, and then finally encounter the esterase and be inactivated.

This more dynamic model provides for greater interaction between the sensillum liquor environment and the receptors because the liquor is seen as coming into play with incoming as well as outgoing pheromone molecules. It places an even greater focus on the kinetics of molecular events, including receptor site interactions, than earlier models, which already heavily emphasized such relationships[39,40]. The model has by no means been universally accepted, however[40,41]. Among the aspects which must be addressed before we can understand which processes are really involved are the in vivo concentrations and kinetics of the pheromone, protein, and enzyme and the proportion of pore tubules in vivo that directly contact the dendrites. As Kaissling pointed out[41], it is on the one hand difficult to envision the binding protein as both a carrier and as aiding in the inactivation of pheromone. On the other hand, there are phenomena, such as the sometimes bimodal return to baseline of receptor potentials, that the non-enzymatic inactivation model fails to explain[41]. In any case, it is now more clear than ever that alterations in any one of several biochemical events in the sensillum could alter the tempo and magnitude of transduction of a split-second pheromone stimulus into nerve impulses, and significantly affect the behavioral outcome.

## *Behavior*

### *Mechanisms*

One of the most fundamental advances in thinking that has occurred in the last ten years has been the realization that the attraction that is induced by pheromone blends is an end-result of actual behavioral reactions[45]. In other words, attraction is a displacement through space, and not a behavior. A corollary is that the flight tracks (the paths taken by the moths through space) are also not behaviors. They, too, are end results of the behaviors. The behaviors, then, are the maneuvers used by the moths, measured on the order of milliseconds, which allow them to control their position both horizontally and vertically. This was spelled out in detail by Marsh, Kennedy and Ludlow[62], but it took some time for the message to sink into the rest of the field.

In order to control its direction and speed of displacement in the horizontal plane, a moth has only two reactions available at any instant: change its course angle (the direction towards which it is thrusting, relative to the

wind line) and change its airspeed (its speed through the air mass next to its body)[62]. The two main mechanisms known to be used for pheromone source location by flying moths, optomotor anemotaxis (steering with respect to the wind) and self-steered counterturning[4,8,49,50], both rely on these two behavioral reactions. The direction of thrust (steering) can be changed by either yawing or rolling the body, the latter having been neglected over the years[9,13,32]. The amount of thrust can be changed either by a change in total wing force (e.g. wing-beat frequency) or by a change in the angle of the body relative to the ground (pitch angle)[31]. The changes in the strength and direction of the wing force in the horizontal plane are inextricably linked to changes in lift (hence altitude) that are also under visual feedback control[31]. Altitude is also controlled by pheromone-stimulated moths[75], and thus places restrictions on the flight in the horizontal plane. Much more needs to be learned about height control in pheromone-stimulated moths[13].

In optomotor anemotaxis, feedback for the control of these reactions is derived visually from the apparent movement of images, especially the ground pattern, over the eyes[31,42,43]. The control of course angle is a steering reaction and control of airspeed is a reaction related to the force of thrust created by the moth's wing movements.

In self-steered counterturning it is not known what external feedback, if any, is employed[31,49,50]. The regularity in the tempo of counterturns both in and out of contact with pheromone[9,93] indicate that there is a motor program underlying these reversals. Thus in the performance of self-steered counterturning, the direction and force of the thrust (course angle and airspeed) may need no external feedback whatsoever. The tempo at which the program runs appears to be set by the concentration at any instant[8,9,49,50].

*Why there are zigzag tracks*

There is now widespread agreement that pheromone-stimulated flying male moths use optomotor anemotaxis to progress upwind toward the source. There has been substantial disagreement, however, as to why the tracks of males flying upwind in a plume have side-to-side deviations back-and-forth across the windline, or zigzags. (Tracks of other species may be said to loop, rather than zigzag[13], but the question of side-to-side deviations remains.) The evidence for most species is that these deviations result from males using self-steered counterturning integrated with optomotor anemotaxis[8,13,50]. There is much support for the existence of counterturning programs in free-flying males of several species[13], for instance the temporal regularity of the lateral deviations[32,93], the continuation of the zigzags even in zero wind[4,32,55] and even the initiation of counterturns in pheromone in zero wind[5]. Optomotor anemotaxis polarizes the otherwise meandering zigzags into an orderly upwind resultant, taking the moth upwind in the plume to the source[5].

Not all moths necessarily integrate counterturning with optomotor anemotaxis while flying upwind in the plume[35], but thus far it appears that all moths do so during casting flight moments after losing the plume[13,49]. Casting flight is not known to occur other than immediately after contact with pheromone, and thus pheromone-mediated flight may be considered to be both flight while in contact, or immediately following contact, with pheromone[13,49], as occurs perhaps hundreds of times during flight within a plume having a fine, filamentous structure[9,49,66]. The zigzag tracks of males not using counterturning while in contact with pheromone may still exhibit some (perhaps less temporally regular) zigzagging due to the moth rapidly losing and contacting the filaments. Thus the male might reiteratively over split-second intervals, begin to go into casting behavior (involving both counterturning and changes in course angle[9] and revert back into positive anemotaxis, in which he attempts to fly straight upwind. For such males this behavior would be similar to that originally proposed for all moths by Kennedy and Marsh[43].

In a recent model explaining zigzag flight in males, counterturning was proposed as not being a factor[76]. Rather the deviations from directly upwind were said to be due to a threshold-related error in the detection of significant transverse image movement in males that use only optomotor anemotaxis to try to steer straight-upwind. Because of the visual error, it was thought that the males repeatedly stray off the windline[76]. However, evidence that runs counter to this model has recently emerged[32,93]. Among the problems with the model was the fact that tethered, rather than free-flying males, were used, preventing unrestricted movement in all three planes of rotation, thus failing to measure lateral flight forces that would have normally occurred during free flight due to rolling[13,32,93]. Another recent model[25], which invoked a form of chemotaxis called transverse klinotaxis[45,49] as a factor in zigzagging flight, has also been refuted[9].

A system which uses counterturning during both upwind flight and during casting may have several functions and advantages over straight-line upwind flight. It involves a continuum from narrow to wide zigzagging[49] that depends on pheromone concentration, and may help facilitate contact with pheromone filaments in the plume, especially during rapid shift in wind direction[9]. Secondly, the counterturning may aid the optomotor anemotactic system in more rapidly detecting changes in off-axis image flow due to shifts in wind direction, which might otherwise be below the visual threshold for a longer period during staight-upwind flight[9,23,50]. Oriental fruit moth, *Grapholita molesta*, males that wing fan while walking toward the source do not counterturn at all, but rather walk in a straight line directly upwind[93]. Because they have contact with the ground, they get their infor-

mation about wind direction from pressure differences across their bodies, and they do not need to counterturn to optimize sensing the wind visually.

*Fluctuating pheromone concentration*

Perhaps the most important recent breakthrough in pheromone research, impacting the fields of neurophysiology, behavior, and biochemistry, as well as our entire understanding of pheromone olfaction, was the behavioral discovery that some male moths need intermittent, not time-averaged, continuous stimulation from pheromone, in order to perform sustained upwind flight. This revelation first emerged in the form of three papers[46-48] which showed that male *Adoxophyes orana* would not progress upwind in a uniform cloud of pheromone, but would readily fly upwind in a point-source plume placed in that same cloud. Something about the plume, conjectured to be fluctuating stimulation created by its fine structure, evoked the sustained upwind flight. These results were confirmed with another species, *Grapholita molesta*[92]. The requirement for intermittent stimulation in *G. molesta* males was then experimentally demonstrated[7] when males failed to zigzag upwind in continuous clouds of pheromone but readily did so when these same clouds were pulsed and interspersed with swaths of clean air.

Wright[95] had earlier pointed out the existence and possible importance of the fine structure of odor plumes to behavior. Murlis and Jones[66] used ionized air and an ion detector to investigate further the nature of structured plumes[67]. However, without the accompanying behavioral evidence[7,46-48,92] that the structure might actually affect the efficacy of the pheromone in evoking upwind flight, Murlis and Jones' important work might well have suffered the same fate as Wright's[95] which was to be cited widely, but its true value overlooked.

*Speed of the reactions to fluctuating pheromone concentration*

It turns out that male moths, whether they are tiny pyralids or huge *Polyphemus* silk moths, usually respond to the loss of pheromone within 0.4–0.5 s by means of a detectable shift from upwind flight to casting flight[9,10,62,63]. Male *G. molesta* have the fastest response to pheromone loss measured in moths thus far, ca 0.15 s[9]. The reaction time (the change to more directly upwind movement) in this species in response to an increase in concentration is equally fast[9]; response latencies of males of other species to an increase in pheromone concentration have not been measured.

The short latencies of response to the onset and loss of pheromone in *G. molesta*, coupled with the knowledge that males flying upwind may contact pheromone filaments only once every second or so[11], led to the realization that perhaps the zigzagging flight tracks of this species are shaped not only by the counterturning and anemotactic systems, but also by the split-second adjustments of these systems to the loss and gain of pheromone[9]. The reiterative change from more directly upwind flight to casting and back again might occur several times each second, and thereby change the width and angles of the track legs, sometimes even resulting in saw-toothed-shaped tracks when the contact and loss occurs regularly according to left and right positions of the moth along its track[9]. Both the anemotactic and counterturning systems in this species have this rapid reaction to fluctuating concentration, whereas adjustments in airspeed occur much more slowly. The knowledge that behavior can change after encounters with single filaments of pheromone has suddenly placed a heavy emphasis on understanding the reaction and recovery speeds of neurons and synapses along sensory pathways (see neurophysiology section below)[39-41]. The new awareness of the rapidity of behavioral reactions has also resulted in a realization that we must focus more on learning about the speeds of biochemical reactions at the receptor and perireceptor levels[39-41,89-91] (see biochemistry section above).

*The importance of pheromone component blend ratios*

Another area of behavioral research which has produced substantial recent progress of profound importance to the field of pheromone research has been in the specificity of males' responses to blends of different quantities and qualities. The primary advance has occurred with recent work by Linn et al.[57,58] who demonstrated experimentally that, as first suggested in experiments by Baker and Cardé[3], males fly upwind to a complete blend of pheromone components at all distances from the source, and not to a hierarchical succession of individual components at different distances[22,37,68,69,82].

It has long been known that the optimum blend ratio of pheromone components is that which most closely approximates the natural ratio emitted by females[24,79]. The recent evidence from both the field and the laboratory which supports the hypothesis that this optimality is due to greater responses to the blend at all distances centers around the fact that males exhibit the lowest behavioral thresholds (are the most behaviorally sensitive) to the full blend of components compared to partial blends or to individual components. In a laboratory wind tunnel using *A. velutinana, G. molesta,* and *T. ni,* both upwind flight and source location were elicited by each species' complete blend of components at 10 to 100-fold lower dosages than were elicited by partial blends[57] (fig. 3). In the field, the complete blend of *G. molesta* components was effective in evoking wing fanning behavior in males at twice the distance from the source than the same dosage of even the best incomplete blends[58] (fig. 4).

What these results mean is that although male behavior can be evoked by a partial blend of components, it takes an inordinately high dosage to do so, much higher than to the complete blend. This fact precludes the feasibility of two scenarios explaining how flight toward the source

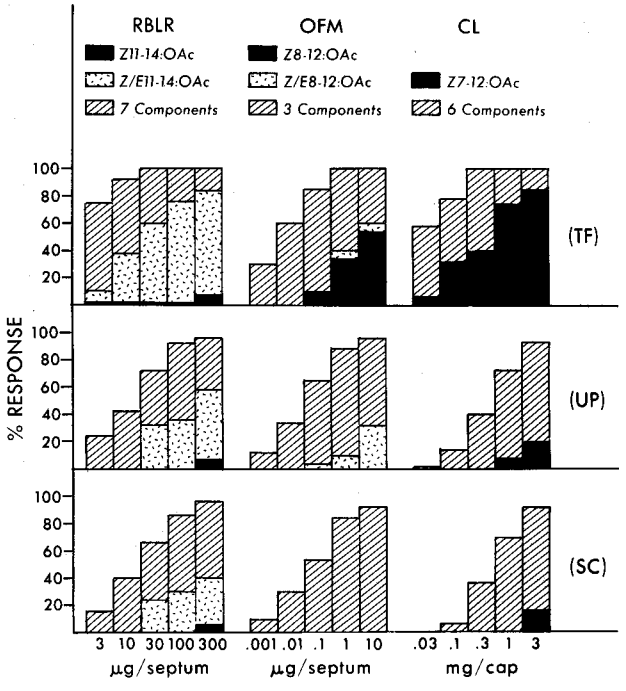


Figure 3. Behavioral responses of three species of moths when exposed to partial blends (solid or stippled bars) or to complete blends of their respective pheromone components in a flight tunnel. RBLR, OFM, and CL refer to redbanded leafroller, oriental fruit moth, and cabbage looper moths, respectively. TF, UP, and SC refer to the behaviors taking flight, upwind flight in the plume, and touching the source, respectively. Note how the threshold for any of the behaviors is always lower for the complete blend than for any of the partial blends tested. Reproduced from Linn et al.[57], by permission of Plenum Press, Inc.

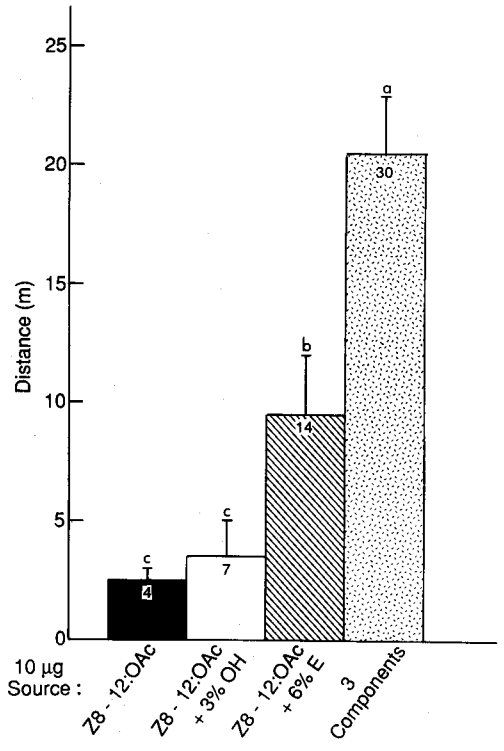


Figure 4. Distance from the source in the field at which quiescent oriental fruit moth males were observed to first exhibit wing fanning while walking, when they were brought close to a source of partial or complete blend of sex pheromone components beginning from over 100 m away. Note how the complete blend evokes wing fanning at the greatest distance, indicating that males have the lowest threshold to this blend. Reproduced from Linn et al.[58], by permission of the American Association of the Advancement of Science.

might occur without the complete blend being involved. In the first scenario, a male sitting or flying far downwind of a pheromone-emitting female (she emits the complete blend) is thought to perhaps be at a distance at which only his receptors specific for the most abundant component would be firing. Hence this component alone would cause upwind flight. The data, however, show if the concentration at this distance is too low for the complete blend to cause upwind flight, then it is definitely far too low for the major component alone to do so.

A key point that must be added here is that these behavioral data[57, 58] do not preclude the possibility that only the receptors specific to the major component might fire at some distance far downwind; the data only remove the possibility that such firing from one cell type is sufficient to evoke behavior. We must be careful to distinguish between the neuronal detection of only one compound in a blend at great distances, and the behavioral response to that single type of neuronal activity at that distance, which is another matter entirely[13]. The former is a virtual certainty, given what we know about emitted blends and the abundance and thresholds of receptor types on antennae (see below), whereas the latter simply cannot happen, according to our best behavioral data thus far[57, 58]. In a second type of scenario, a flying male might intersect a female-emitted plume at a distance close to the source at which the concentration of a partial blend is above the behavioral threshold for upwind flight. Of course, the data show that the concentration would also be even farther above threshold for the complete blend, and so again the complete blend will be responsible for any upwind flight which occurs.

As for its effect on neurophysiological research, this behavioral framework, centered on the importance of the complete blend, naturally places an emphasis on understanding the integration of neuronal activity following the response to the individual components, and not merely analyzing the response thresholds and dosage-response curves of isolated, component-specific pathways. This change in perspective is already leading to advances in understanding odor-quality encoding at the neuronal level (see next section).

## *Neurobiology and behavior*

### *Pheromone blend quality*

There is a unanimity of agreement that the processing of information concerning the ratio of components in the airborne blend begins at the periphery, when pheromone contacts the long sensilla trichodea (sensory hairs) cover-

ing a moth's antennae and antennal neurons generate action potentials[39-41]. There has even been a somewhat recent general concensus that antennal neurons sensitive to pheromone components have an extraordinarily high degree of specificity for just one component, even though some earlier studies appeared to show some heterogeneity of receptor site types on the same cells with a corresponding possibility for integration of blend quality at the receptor cell level[70,71]. Among the reasons as to why some earlier studies are now thought to be flawed[1] is the likelihood that the samples of pheromone components used to stimulate the cells may have been contaminated with trace amounts of other components[1]. Some evidence persists that shows higher activities of single antennal neurons in response to blends than would be predicted from the activities of the cells in response to the single components presented alone[72,73]. The possibility of integration of odor quality information at the periphery thus should be kept in mind in formulating models for pheromone olfaction in moths.

The high degree of specificity of antennal neurons to different pheromone components is best illustrated by the turnip moth, *Agrotis segetum* (Noctuidae)[86] (fig. 5). It can be seen that the 'A' type cells have extreme specificity, each cell tuned mainly only to one of the three known pheromone components of this species, (*Z*)-5-decenyl acetate, (*Z*)-7-dodecenyl acetate, or (*Z*)-9-tetradecenyl acetate (*Z*5-10:Ac, *Z*7-12:Ac, and *Z*9-14:Ac, respectively). The relative abundance of each cell type is quite different, with nearly 70% of the sampled cells being tuned to *Z*5-10:Ac, ca 28% tuned to *Z*7-12:Ac, and only 2% tuned to *Z*9-14:Ac[86] (fig. 5). The presence of all three components in the blend is crucial to sustained upwind flight and source location. The 'B' type cells housed within the same sensory hairs as the A cells do not respond to the pheromone components, but do respond to a known behavioral antagonist, (*Z*)-5-decenol, and (*Z*)-8-dodecenyl acetate, the major pheromone component of *G. molesta* and some other olethreutine moths[83].

How the processing of pheromone quality proceeds after the antennal neuronal level, however, continues to be a matter of disagreement. On the one hand, there is 'across-fiber patterning'[33] or some sort of deutocerebral or protocerebral integration of the more peripheral inputs, as a way that pheromone quality is processed[20,21,33,41,71]. On the other hand, there is the possibility that, in essence, the quality is not processed but the responses from each component-specific cell evokes a different stage of behavior[25,37,68,69,82]. The behavior might be in different forms (e.g., flight, landing, copulatory thrust), or else related to the distance from the source such that threshold and adaptation-related 'tuning' features of each cell type would cause them to respond at their own optimum distance and aid in source location[82]. Combinations of the two models also exist, where both behaviorally and neurophysiologically a hierarchical succession of single components or blends would

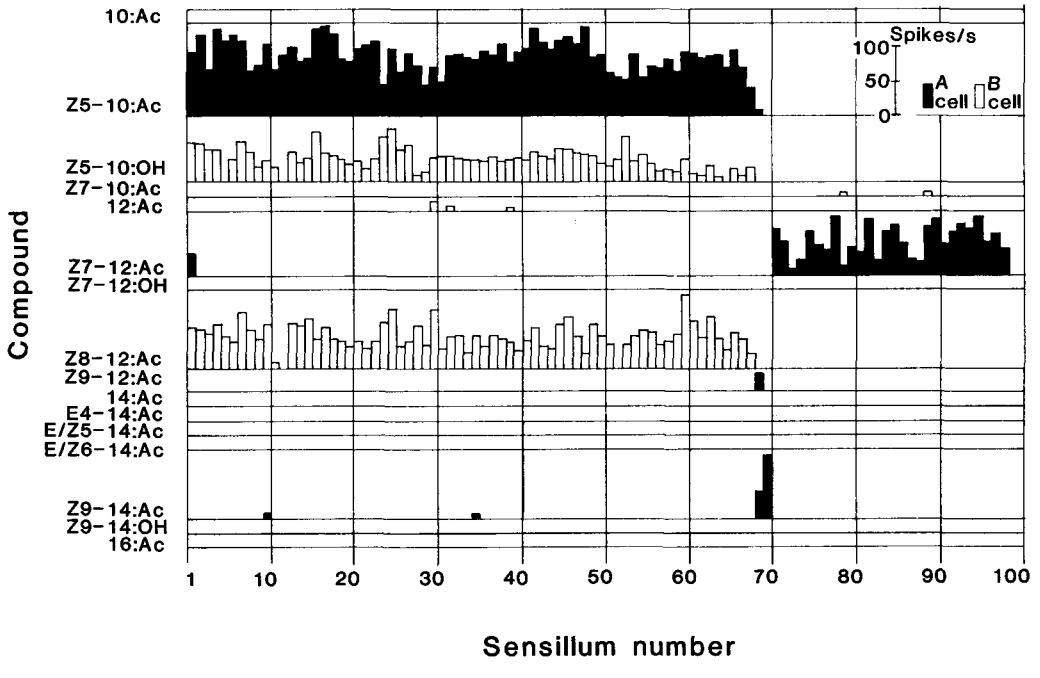


Figure 5. Response spectra of antennal neurons in the sensilla of males of the turnip moth, *Agrotis segetum*, to its three sex pheromone components, *Z*5-10:Ac, *Z*7-12:Ac, and *Z*9-14:Ac, and other pheromone-like compounds. Each sensory hair contains both an A and a B neuron, whose action potentials have a characteristic amplitude which allows the researcher to discern which cell is firing and at what frequency. Note that the A cells fire only in response to the three pheromone components, and that the cells sensitive to each component are housed in separate hairs. When B cells are responsive at all, on the other hand, it is when two other compounds are puffed over the cells, and both compounds are known antagonists of behavior. Reproduced from Van der Pers and Löfstedt[86], by permission of Clarendon Press.

control the behavior, sometimes beginning from just one component and working up to increasing numbers of components[68, 69, 82].

The two major problems with non-across-fiber pattern explanations both involve a failure to understand the behavior, such as we know it. First is the fact that the only unequivocal behavioral evidence that exists thus far shows that individual components do not act alone or in partial blends to evoke behavior[3, 57, 58]. A second problem is that most of these explanations fail to even mention anemotaxis as a behavior evoked by individual compounds or blends. Thus by default they give unnecessary attention to a need for the cells to 'code' for concentration and hence for distance from the source, and imply, since they have listed no other mechanism, that such information about distance is essential to keep the males progressing toward the source. Generally neglected is the fact that flying moths steer with respect to the wind, not to the chemicals (see Behavior section above).

Negation of the across-fiber patterning hypothesis should not and does not come from a failure to find integration of blend quality information occurring at the antennal neuronal level[1]. Likewise, a failure to find blend-specific cells at the deutocerebral level does not necessarily support the component-hierarchy behavioral models; cells that respond optimally to blends could merely be located at higher levels[19–21], much as color-coded cells occur very far up the line in vertebrate visual systems[96].

A problem with the across-fiber pattern model, although much evidence supports it, is that the actual type of pattern is usually not described, only that the pattern would be optimal and unique to the pheromone blend. It seems logical though, that the blend quality, i.e. the ratio of components in the airborne blend, is coded by simple ratios of action potentials from the different component-specific receptors[41]. Until recently, there has been no combined behavioral and single cell data for pheromone quality processing. Direct and specific support for this model now comes from recent data from Akers and O'Connell[1] using the redbanded leafroller moth, *Argyrotaenia velutinana*, coupled with previously published behavioral data from this same species[2]. In recording from single antennal neurons, Akers and O'Connell found that in each of the sensory hairs they sampled were two neurons, one specific for (*Z*)-11-tetradecenyl acetate, the other specific for the opposite isomer, (*E*)-11-tetradecenyl acetate. These are the two pheromone components that have the greatest effect on behavior in this species[16]. Behavioral results had indicated that males respond optimally in wing fanning assays (and numerous field studies involving flight to the source) to the natural female-emitted *E*:*Z* ratio of 92:8. Wing fanning diminished severely when this proportion strayed by just a few percentage points to either side of the 8% optimum[2] (fig. 6). When Akers and O'Connell puffed a series of blends over any of the hairs, both cell types appeared to respond according only to the absolute concentration of the isomer to which it was sensitive, and not to the blend. They, therefore logically ruled out that any single sensory neuron could code for odor quality, which corrected a previous study[71].

The response functions of the two cells to the blends (actually to the absolute concentration of the particular isomer to which they are sensitive, irrespective of blend ratio) are quite simple (fig. 6). The 'A' cell has a flat response curve because the concentration of *Z* isomer was held constant in these blends, with only the amount of *E* isomer being altered. The 'B' cells (sensitive to the *E* isomer) exhibit an increase in firing as the concentra-

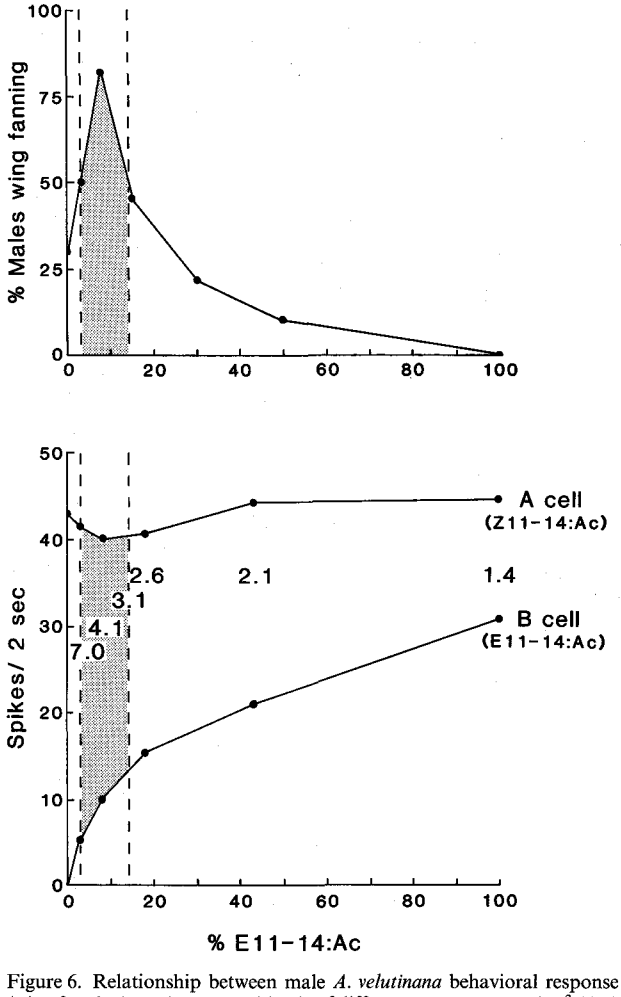


Figure 6. Relationship between male *A. velutinana* behavioral response (wing fanning) to pheromone blends of different component ratios[2] (top) and the firing rates of antennal receptors specific for the same two components when they were exposed to approximately the same series of two-component ratios[1] (bottom). The A cell responds preferentially to (*Z*)-11-tetradecenyl acetate, the concentration of which was held constant at 10 ng in each blend, whereas the B cell responds preferentially to (*E*)-11-tetradecenyl acetate whose concentration (and proportion in the blend) was made to increase (from left to right in the figures). The region of blends that evoked wing fanning in 50% of males is superimposed on the receptor activity to show that the ratio of impulses from cell A to those from cell B (bold numbers) changes the quickest and passes through an optimal value of 4:1 in the region where discrimination of blend ratios is the best.

tion of *E* in the blend increases. But interestingly, the slope of the rise is steeper in response to very slight increases in the concentration of *E* (left-hand portion of the curve), than with larger increases (right-hand portion of curve) (fig. 6). The left-hand (low-*E*) blend region is the one in which males best discriminate changes in the *E:Z* ratio (absolutely, not comparatively) according to the wing fanning data, because the change in behavioral response per unit change in blend ratio is greatest here. Similarly, the greatest change in the ratio of A to B receptor impulses (centered about an optimal 4:1) per change in *E:Z* blend ratio occurs precisely in this same region. In the region to the right of 20% *E* (fig. 6) where the amount of *E* approaches that of *Z*, the ratio of receptor firing, now down to 2:1 or less, also changes more slowly, as do differences in behavior.

In other sensory systems designed for stimulus quality processing (e.g., color vision), behavioral discrimination is best where the receptor firing ratios change the most per unit change in stimulus quality. The most pronounced behavioral discrimination would occur, for instance, where the tuning curves of two receptor types have the greatest change in slope differential, especially when the slopes are steepest and in the opposite directions[65] (see fig. 3 of Menzel et al.[65]). In the *A. velutinana* pheromone component system, the change in the receptor firing ratio would be accentuated in the low *E* region if the slight dip in the A cell response function (fig. 6) were real, and not just due to experimental variation. However, even with a flat A cell curve, the greatest blend discrimination will be in this region because the slope differential between the two receptor types remains the greatest here.

Although this model for pheromone blend quality encoding would be not different from those of some other sensory systems of other modalities, such as color vision, there exists no known predictable spectrum of odor molecules. The sensations of odor might be best considered to be analogous to those for extra-spectral colors such as purple, which do not exist on the continuum of single wavelengths of light.

*Blends and the enhancement of pheromone intermittency*

The above ratio model does not necessarily depend on whether the CNS integration of the A and B inputs involves excitatory post-synaptic potentials for both the A and B inputs or excitatory inputs from one cell type, A, let's say, and inhibitory inputs from B. There is recent evidence, however, that at least one aspect of the processing of odor quality at the deutocerebral level, enhancement of intermittent deutocerebral output in response to pheromone filaments, involves both excitatory and inhibitory inputs from the periphery[26,28]. In the tobacco hornworm moth, *Manduca sexta*, the onset and offset of action potentials during pheromone stimulation from neurons that send their output to the protocerebrum in the antennal lobe, is accentuated significantly when a blend of two components, bombykal plus a minor component, is used for stimulation and not just bombykal alone. In some cases the minor component evokes inhibitory slow potentials in these deutocerebral cells, which both reduces the background firing just before the onset of excititory potential from bombykal and also causes the action potentials in response to bombykal to subside much more quickly following the pulse of the blend than would have occurred had only bombykal been puffed[26,28]. In other cells the components switch functions, and it is bombykal which evokes the inhibitory slow potentials.

As pointed out by Hildebrand and Montague[36], there is an abundance of GABA, an inhibitory neurotransmitter, in the macroglomeruli of the antennal lobes of *Manduca* with the potential for lateral inhibition. It is in the macroglomeruli where the first integration of the pheromone signal takes place[26,27,36]. Thus, the blend of components appears to actually enhance filament-caused intermittency, and since such intermittency has been shown to optimize sustained upwind flight (see above), this neuronal effect is of no small consequence to flying males. One could not dream of a more direct effect of blend quality on attraction. If the blend ratio strayed to the low end and contained too little *E* isomer, the deutocerebral output in response to rapidly arriving plume filaments might be too smooth under some conditions. On the other hand, should the blend contain an excessive proportion of *E*, the action potential output from the deutocerebral cells might be excessively inhibited, although sufficiently intermittent, resulting in the overall spike frequency being too low to sustain upwind flight.

In other species, there appears to be a variety of deutocerebral cell types. In *Heliothis virescens* and *H. zea*, as in *Manduca*, some cells respond intermittently to intermittent stimulation and their intermittency is enhanced when the blend of components is presented[29]. However, there are other cells that respond only to single compounds, their activity not enhanced at all by a blend, and these cells respond tonically to intermittent stimulation[29]. It thus seems possible that such neurons merely amplify (due to convergence of antennal neurons[19]) the inputs from their respective component-specific antennal neurons before sending them to higher-order cells that are optimally responsive to blends.

*Sensory adaptation and blend quality*

Recently, there has been evidence that provides a link between sub-optimal upwind flight in the plume and conditions that interfere with antennal neurons' abilities to respond to rapid plume filament-caused fluctuations. Males of the turnip moth, *Agrotis segetum*, change from upwind flight to in-flight arrestment (station-keeping) in response to an excessively concentrated, 300-µg plume of its three components (see above)[60]. No such arrestment is observed when males fly upwind in plumes from 3- or 30-µg sources. Recordings from antennal neurons ex-

posed to these same plumes 70 cm downwind of the source revealed that the firing rates from cells sensitive to (*Z*)-5-decenyl acetate decreased to near zero (adapted) within about 5 s after being placed in the 300-µg plume, whereas such adaptation was not observed when these same neurons were placed in the 3- or 30-µg plumes [12]. In plumes from the 300-µg source, the cells appeared unable to recover sufficiently from the arrivals of successive high-concentration filaments in order to adequately fire in response to subsequent filaments. The biochemical machinery of the sensilla appeared to be unable to handle these concentrations and clear away pheromone fast enough such that the receptor cells had sufficient time to disadapt [12, 82].

Interestingly, 30 out of 32 of the (*Z*)-5-decenyl acetate-sensitive neurons adapted in the 300-µg plume, but only 5 out of 12 neurons sensitive to a second component, (*Z*)-7-dodecenyl acetate, adapted when placed in these same 300-µg plumes. Thus a moth flying upwind in the 300-µg plume may not only experience the sensation of an odor decrease when in fact there was none, but also the sensation of blend quality may change due to the decrease in the ratio of firing of cells sensitive to (*Z*)-5-decenyl acetate compared to those firing in response to (*Z*)-7-dodecenyl acetate [12]. The relationship between odor quality and quantity, and specifically the ratios of firing rates of component-specific receptor cells being altered at excessive concentrations, were all anticipated by Kaissling [41].

In *G. molesta*, partial adaptation of antennal neurons sensitive to (*Z*)-8-dodecenyl acetate was observed when the cells were challenged by rapid pulses of the complete pheromone blend. Adaptation was facilitated when the cells were chilled by about 6 °C, and was characterized by an attenuation and reduction of the cells' fluctuating output in response to 2/s pulses [12]. Adaptation occurred at the warmer temperature only when the cells were challenged with 3/s or higher pulse frequencies. As in *A. segetum*, the adaptation appears related to an overloading or swamping of the biochemical machinery of the sensilla and receptor cells by excessively concentrated and rapidly arriving plume filaments. Under other conditions, the sensilla would otherwise be able to adequately register and clear away pheromone in order to get the receptors ready to accurately respond to the next-arriving filament. Cool temperatures would slow down the biochemical processes involved whereas the airborne filaments would continue to arrive on the antenna just as rapidly as at warmer temperatures. Interestingly, in flight tunnel experiments, males became prematurely arrested in mid-flight more frequently in response to higher concentrations of pheromone at cooler temperatures compared to temperatures that were 6 ° higher [59]. Moreover, at higher concentrations and cooler temperatures, the specificity of the upwind flight response to blend ratios now shifted to blends emitting lower proportions of the *E* isomer. Even the natural 6% *E* blend became deficient in evoking sustained upwind flight, and 2% *E* became optimal under these conditions.

These results with *G. molesta* are again consistent with an adaptation-induced skewing of the neuronal ratio coding for blend quality [41]. The fluctuating output from antennal neurons sensitive to the *Z* isomer would become reduced relative to cells sensitive to the *E* isomer. For the latter cells, the same plume filaments strike the antenna simultaneously and with the same frequency as for *Z*-sensitive cells, but the concentration of the *E* isomer is nearly 20 times lower than *Z*. Thus, following adaptation of *Z*-sensitive cells, the ratio of action potentials [41] transmitted to higher-order neurons would be too heavily weighted in favor of *E*-sensitive cells when in fact the actual ratio of molecules of *E* and *Z* had never changed. Behaviorally, blends with 2% *E* would now become optimal at sustaining upwind flight. Interestingly a similar shift in response specificity at higher emission rates in a wind tunnel appears to occur in the light brown apple moth, *Epiphyas postvittana* (Muggleston and Foster, unpublished). At higher concentrations males exhibit greater levels of sustained upwind flight in response to blends containing lower ratios of the diene component than they do at lower concentrations. Again the diene is a minor component (less than 10% in the natural blend relative to the monoene), and thus the swamping-induced adaptation of the cells sensitive to the most abundant component in the airborne blend (the monoene) would explain such a shift [12].

*Integration of odor- and vision-related neuronal activity*

In order for an airborne male moth to perform pheromone-induced optomotor anemotaxis, stimulation from pheromone must be integrated at some level with feedback about motion from the visual system [50]. Such integration is in evidence in the multimodal cells discovered by Olberg [74] in the neurons descending from the brain of *Bombyx mori* and heading toward the pterothoracic ganglia. Olberg found cells which changed their firing frequency from either a high to low state (or vice-versa) in response to successive pulses of pheromone. Some of these same 'flip-flopping' interneurons also changed states when a light was switched on or off. Some cells responded only to pheromone fluctuations, while others responded only to changes in the visual field. Others were multimodal, or stimulated by different modalities such as odor and visual stimuli. Interestingly, some of the flip-flopping cells responded to the transverse movement of images across the eyes [74]. The close association of cells responsive to such movement – precisely the kind which would be used by insects to steer in compensation for wind-induced drift [42, 50] – and fluctuating pheromone stimulation, appears to provide solid evidence for the neuronal underpinnings needed in order for moths to sustain their upwind flight toward conspecific females. Multimodal cells appear to have their origin in the protocerebrum [27, 56].

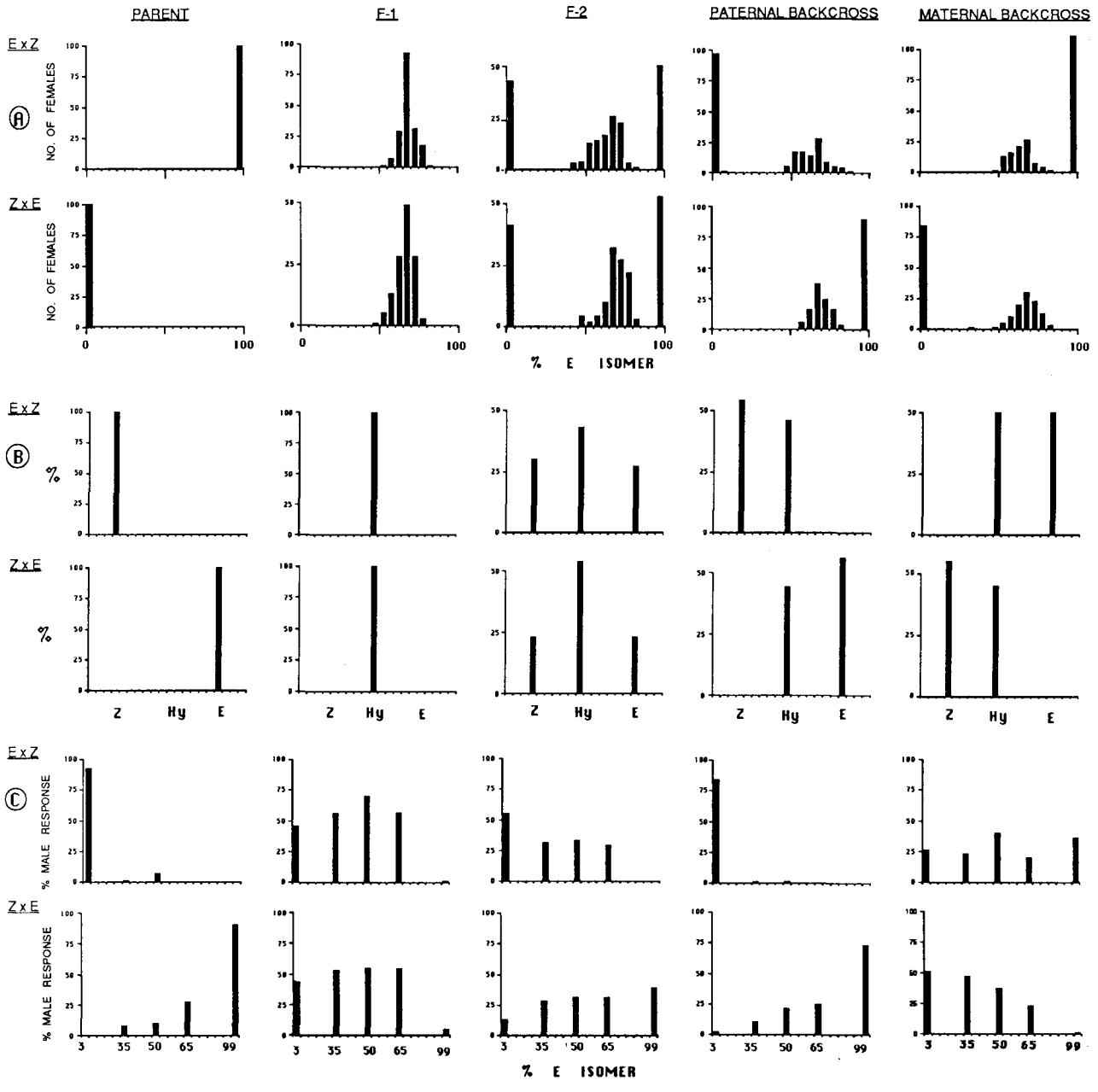


Figure 7. The pattern of inheritance of: (*A*) sex pheromone component blend ratios from female glands; (*B*) spike amplitudes of antennal neurons specific for the two components; and (*C*) specificity of the upwind flight response in a wind tunnel to two-component blend ratios, in the European cornborer moth *Ostrinia nubilalis*[81]. The patterns for both pheromone production and spike amplitude of receptors (*A* and *B*) are not significantly different from that expected from simple Mendelian inheritance involving one autosomal gene with two alleles. However, for the specificity of upwind flight (*C*), the paternal backcrosses are definitive in revealing that a single sex-linked gene with two alleles is responsible for male response specificity. The progeny from these backcrosses behaved identically to their fathers, with the *Z*-strain fathers and backcross sons flying upwind only to the 97% (*Z*) blend, and the *E*-strain fathers and backcross sons preferentially flying upwind to blends containing predominantly (*E*)-11-tetradecenyl acetate. Reproduced from Roelofs et al.[81] by permission of the National Academy of Sciences Press.

## *Genetics and evolution*

### *The Ostrinia nubilalis pheromone system*

New knowledge has recently been acquired concerning the inheritance of the three major aspects of pheromone communication discussed above: pheromone production, reception, and behavioral response. The information has come in a remarkable study on the European cornborer, *Ostrinia nubilalis* (Pyralidae), by Roelofs et al.[81]. They concluded that the major phenotypic aspects of both production and reception are inherited as single autosomal genes with two alleles; the production and reception genes are not closely linked, however[34] (Löfstedt et al., unpublished). Remarkably, specificity of

behavioral response to blends, as measured by upwind flight in a plume, is inherited not autosomally, but as a single, sex-linked gene, again with two alleles. Thus, none of the three major determinants of successful sex pheromone communication are linked, and in males, the genes controlling receptor types are on autosomal chromosomes whereas the specificity of response to blends that the receptors' firings evoke is on the sex chromosomes.

The new data on pheromone production confirmed and extended the findings of a previous study [52]. Most of the *E*-strain cornborers in the U.S., very likely originally imported from Italy [51], emit 97–99% (*E*)-11-tetradecenyl acetate and 1–3% (*Z*)-11-tetradecenyl acetate. Females of the *Z* strain, originating most likely from somewhere in the rest of Western Europe, emit 97–99% *Z* isomer and 1–3% *E* isomer [81]. Hybrids emit a 65:35 blend of *E*:*Z* (fig. 7A). The data from reciprocal crosses, F2 progeny, and the backcross progeny indicate clearly that the observed pheromone ratios are no different from that expected if a single autosomal gene with two alleles was controlling the blend ratio (fig. 7A).

The remarkable data on the receptor specificity was made possible by the discovery that of the two pheromone-component-sensitive cells in each sensillum, the cell giving the larger-amplitude spike in the *Z*-strain males was always the cell sensitive to the *Z* isomer, and in the *E*-strain males the cell with the larger spikes was always the one sensitive to the *E* isomer [34]. In hybrids both the A and B cells gave spikes of equivalent heights, and thus male receptor phenotypes could be accurately and quickly revealed by means of one puff each from the *Z* and *E* isomers. Using the same reciprocal crosses and backcrosses as for female pheromone production above, the observed pattern of inheritance was shown to be quite similar to that of females, and did not differ significantly from that expected where the inheritance is controlled by a single autosomal gene with two alleles (fig. 7B) [81]. Further work was performed (Löfstedt et al., unpublished) in which F1 hybrids were created by crossing *E*-strain females with *Z*-strain males and then performing backcrosses of these intermediate-type males with females of the *E*-strain. *E*-typ males from this backcross were then selected from among the progeny by removing one antenna and checking for a large spike to a puff from the *E* isomer. These *E*-type males were then mated with pure *E*-strain females, and when some female progeny produced intermediate ratios of the *Z* and *E* isomers, not 97% *E* isomer, then it was shown that production and reception, though both autosomally controlled, are not very closely linked.

In the wind tunnel tests of inheritance of behavioral upwind flight responses to the various *E*-*Z* ratios, the F1 backcross results quickly revealed a pattern quite different from those of production and receptor specificity (fig. 7C). The male progeny from the paternal, not maternal, backcrosses back to either the *Z* or *E* strain appeared to respond identically to the males from that pure parental strain [81] (fig. 7C). This striking result demonstrated that the observed inheritance of specificity of upwind flight to blends of *E* and *Z* is not significantly different from what would be expected were the behavioral response controlled by a single sex-linked gene with two alleles. Thus, the behavior is controlled by a gene that is not even on the same chromosome as the gene for receptor cell component specificity.

Using our recent knowledge about upwind flight behavior and the specificity of response, we can guess that the sex-linked gene does not code for some special difference in the anemotactic and self-steered counterturning systems. Rather, the specificity would appear to lie in the CNS processing of the antennal neurons' outputs, perhaps beginning at the deutocerebral level. Even the receptor cells cannot yet be ruled out, because the action potential frequency output in response to various blends has not been investigated; only the genetics of spike size has been determined, and behavior should result from relative spike frequencies, not size. Such recordings need to be performed. It would also be informative for deutocerebral and protocerebral recordings to be performed to search for such blend-specific integration that switches on both the anemotactic and counterturning programs.

## *Conclusion*

Recent progress in understanding the major facets of sex pheromone communication in the Lepidoptera has been great. The seemingly hopeless diversity of pheromonal signals and great complexity of behavioral responses have been demonstrated to be under the influence of a relatively limited array of underlying biochemical, neurobiological and behavioral mechanisms. In addition, the genetic control of these fundamental mechanisms is also beginning to be understood, which opens the door to the future isolation of the responsible genes. It is thus with great optimism that researchers are continuing to forge ahead in the field of lepidopterous sex pheromones, an area that holds perhaps unparalleled possibilities for gaining knowledge relevant to the evolution of sexually reproducing organisms and for applying this knowledge to the benefit of natural and agricultural environments.

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